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In re Application of:

Ullrich et al.

Art Unit: 1614

Application No. 09/600,826

Examiner: J. F. Murphy

Filed: September 7, 2000

For: USE OF INHIBITORS FOR THE
TREATMENT OF RTK-
HYPERFUNCTION-INDUCED
DISORDERS, PARTICULARLY
CANCER

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AMENDMENTS TO SPECIFICATION
MADE VIA SUPPLEMENTAL PRELIMINARY AMENDMENT

Amendments to the paragraph beginning at page 14, line 16:

FGFR-4^{388Arg} and FGFR-4wt were amplified by the PCR reaction. For this, the following primers were used: sense-GCTCAGAGGGCGGGCGGGGGTGCCGGCCG [SEQ ID NO: 3]; anti-sense CCGCTCGAGTGCCTGCACAGCCTTGAGCCTTGC [SEQ ID NO: 4]. For the PCR reaction, the following were used: 1.5 U/25 μ l Expand-Polymerase (Boehringer, Mannheim) and reaction buffer according to the manufacturer's instructions: 200 μ M dNTP's; 0.01% v/v Triton X100; 10% v/v DMSO, and 0.2 pmol each of sense and α -sense primer. The following reaction steps were performed: 35 cycles, 94°C 1 min, 64°C 1 min, 72°C 2.5 min. MDA-MB-453 cDNA was used for the cloning of FGFR-4^{388Arg}, and K562 cDNA for the cloning of FGFR-4wt. The PCR products were cloned in the pcDNA3 vector (Invitrogen). In this way, both a FGFR-4 with the G388R and also a wild type FGFR-4 could be obtained for further tests.

Amendments to the paragraph from page 14, line 27, through page 15, line 2:

Amplification of the transmembrane domain of FGFR-4. The following primers were used: sense-GACCGCAGCAGCGCCCGAGGCCAG [SEQ ID NO: 5]; anti-sense AGAGGGAAGAGGGAGAGCTTCTG [SEQ ID NO: 6]. For the PCR reaction, the following were used: 1.5 U/25 μ l Taq-Polymerase (Boehringer, Mannheim) and reaction buffer according to the manufacturer's instructions: 200 μ M dNTP's; 0.2 pmol each of sense and α -sense primer, 0.5 μ l cDNA or genomic DNA from tumour biopsies and cell lines; the following reaction steps were performed: 35 cycles, 95°C 45 secs, 72°C 45 secs.

Amendments to the paragraph beginning at page 15, line 15:

Gentotype analysis of genomic DNA by restriction digestion.

Genomic DNA from the tissue samples of the primary tumours was isolated by standard methods (Current Protocols in Molecular Biology, John Wiley and Sons, Inc., 1995). In order to be able to genotype analyse the genomic DNA, the transmembrane region in the FGFR-4 gene was amplified with the following primers in a PCR reaction: 5'-GACCGCAGCAGCGCCCGAGGCCAG-3' (bp 1129-1142; [SEQ ID NO: 5]), and 5'-AGAGGGAAGAGGGAGAGCTTCTG-3' (bp 1275-1297; [SEQ ID NO: 6]). For the PCR reaction, Ready-to-Go PCR Beats (Pharmacia, Uppsala, Sweden) were used. The following PCR cycles were used: 3 min at 95°C, 45 secs at 94°C, 45 secs at 72°C and 5 mins at 72°C. A total of 35 cycles were performed. The PCR products were incubated for 1 hr at 60°C with 5 U/25 µl of BstN1 (NEB, Schwalbach/Taunus). The DNA fragments from the restriction digestion were separated with a 20% polyacrylamide gel and stained with ethidium bromide. The ³⁸⁸Arg allele is characterized by two fragments of 80 and 29 bp size, while the ³⁸⁸Arg allele is indicated by a single 109 bp sized fragment. Each genotype analysis was repeated three times.

Amendments to the paragraph from page 15, line 30, through page 16, line 8:

DNA sequencing of PCR products. For the sequence analysis of the transmembrane domain of FGFR-4, the PCR products were cloned into the Bluescript vector. For this, a PCR reaction was performed as already described. The following primers were used: sense-GGGAATTTCGACCGCAGCAGCGCCCGAGG [SEQ ID NO: 7]; α-sense-GCTCTAGAAGAGGGAAGAGGGAGAG [SEQ ID NO: 8]. The PCR products of the cloning of FGFR-4^{Arg388}/wt could be directly sequenced in the vector pcDNA3. The DNA sequencing of plasmid DNA was performed by the chain termination method. After annealing of the T/-primer onto the plasmid DNA, the sequencing reaction was performed with T/-DNA polymerase (Pharmacia, Freiburg). The products of the sequencing reaction were then separated on a denaturing 5% polyacrylamide gel (7.5 M urea; 1 x TBE) and exposed on Xray film after drying (see Fig. 3). From this, the DNA sequences of the wild type and also of the mutation, were obtained.